IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS AND INTERFERENCES

In re:

Serial No.: 09/762,376

Inventors: Wong, et al.

Filed: 07/20/2001

Title: α2,8/2,9 Polysialyltransferase

Examiner: Rao, Manjunath N.

Art Unit: 1652

Appeal Brief

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The Applicant appeals the Office Action, dated 03/23/2004, and the final rejection of claims 7-9 therein.

Real Party in Interest

The present application has been assigned by all inventors to the Scripps Research Institute, which is the real party in interest.

Related Appeals and Interferences

There are no related appeals or interferences.

Status of Claims

Claims 1-6 are canceled.

Claims 7-9 are pending.

Claims 7-9 are rejected.

Claims 9 is objected to.

Claims 7-9 are on appeal.

Status of Amendments

No amendments have been filed subsequent to the final rejection.

Summary of Invention

The invention is directed to a two step cell-free enzymatic synthesis of a polysialic acid product having alternating α2,9 - and α2,9 linkages of sialic acid. In the first step, a sialic acid acceptor and a CMP-sialic acid donor is contacted under cell-free conditions with α2,9 - and α2,9 polysialyltransferase for polysialylating the sialic acid acceptor, releasing CMP, and forming the polysialic acid product. (Specification, page 4, first paragraph) In the second step, the CMP is removed using alkaline phosphatase. (Specification, page 16, first sentence)

Issue

There is only one issue, viz., are claims 7-9 patentably obvious under 35 U.S.C. §103(a) over Steenbergen et al., (J. Bacteriol. 1992, vol. 174(4): 1099-1108) or Vann, (FEMS Microbiology Lett., 1995, vol. 128(2): 163-166) in view of Van Dijk et al., (Analytical Biochem., 1981, vol. 117(2): 346-353).

The Examiner's objection to claim 9 regarding the improper dependency is not being appealed.

Grouping of Claims

Claims 7-9 share a common inventive step and all stand or fall as a group according to the issue of obviousness.

Arguments

Arguments:

Claims 7-9 have been finally rejected under 35 U.S.C. §103(a) as obvious over Steenbergen et al., (J. Bacteriol. 1992, vol. 174(4): 1099-1108) or Vann, (FEMS Microbiology Lett., 1995, vol. 128(2): 163-166) in view of Van Dijk et al., (Analytical Biochem., 1981, vol. 117(2): 346-353). Applicant traverses and appeals this basis for rejection.

Claims 7-9 are directed to a two step cell-free enzymatic synthesis of a polysialic acid product having alternating α2,9 - and α2,9 linkages of sialic acid. In the first step, a sialic acid acceptor and a CMP-sialic acid donor is contacted under cell-free conditions with α2,9 - and α2,9 polysialyltransferase for polysialylating the sialic acid acceptor, releasing CMP, and forming the polysialic acid product. In the second step, the CMP is removed using alkaline phosphatase.

Steenbergen discloses that *Escherichia coli* K92 synthesizes a sialyl α2,9- α2,9 linked polymer; Steenbergen discloses an initial molecular description of K1 and K92 sialyltransferase and a partial purification of K92 sialyltransferase within a membrane bound fraction; Steenbergen discloses that K92 sialyltransferase has sialyltransferase activity, but does not disclosed that K92 sialyltransferase has **poly**sialyltransferase activity. Steenbergen discloses a suggestion, supported by genetic evidence only, that a single gene product in *Escherichia coli* K92 is capable of synthesizing a polysialyl

α2,9- α2,9 linked polymer. However, Steenbergen stops short of actually disclosing that K92 sialyltransferase is, by itself, a **poly**sialyltransferase capable of synthesizing a polysialyl α2,9- α2,9 linked polymer. Steenbergen was unable to claim a disclosure that K92 sialyltransferase is a **poly**sialyltransferase because Steenbergen failed to purify K92 sialyltransferase to homogeneity (see Vann, page 163, second column, footnote 12 citing the Steenbergen reference).

Vann discloses a filter assay for partially purified membrane bound protein fractions containing polysialyltransferase activity. Vann also compares his filter assay with the paper chromatography assay disclosed by Steenbergen (Vann, page 166, first column, footnote 12 citing the Steenbergen reference). Indeed, Vann teaches that Applicant's invention had not then been discovered. The Abstract for the Vann reference states the following:

> "An understanding of how polysialyltransferase functions in the synthesis of polysialic acid **will** require enzyme purification and characterization in concert with genetic analysis." (Vann, abstract, second sentence.)

Accordingly, Vann teaches that polysialyltransferase had not yet been sufficiently purified so as to characterize its function with respect to the synthesis of polysialic acid. Vann does not disclose Step A of Applicant's claim 7. Vann teaches that Step A of Applicant's claim 7 was unknown at the time of that reference.

Van Dijk discloses an assay for CMP-sialic acid hydrolase. The assay employs alkaline phosphatase to release phosphate from CMP. Van Dijk does not teach that alkaline phosphatase would be useful for releasing phosphate from CMP in connection with a synthetic process for producing a polysialic acid product having alternating α2,9

- and α2,9 linkages using a polysialyltransferase. Indeed, as indicated below, this would be an improbable suggestion.

The present invention is enabled by the development and use of a modified polysialyltransferase. This polysialyltransferase is most clearly shown in Figure 2b of the specification of the present application and is labelled "membrane protein". The "memberane protein" of the present invention is distinguished from the "membrane bound α2,9/α2,9 polysialyltransferase" in Figure 2b by the addition of a hexomeric hystidine at the amino end. Figure 1 illustrates the plasmid containing the "membrane protein" with the hexomeric hystidine. The specification discloses the "membrane protein" with the hexomeric hystidine at page 4, paragraphs 2 and 3.

The full length form the protein α2,9/α2,9 polysialyltransferase of the present invention remains mostly in the membrane-bound form. However, about 30-40% of the protein is released and this released protein retains full catalytic activity and is the kernel of the present invention.

The DNA plasmid disclosed in Steenbergen does not encode the membrane protein of Figure 2B having a hexomeric hystidine at the amino end. There is no teaching by the Steenbergen reference that any fraction of the K92 sialyltransferase is released from the membrane fraction or that such released fraction would retain full catalytic activity.

The present application teaches, for the first time, that α2,9/α2,9 polysialyltransferase is capable, without assistance from another membrane enzyme, of catalyzing a cell-free enzymatic synthesis of a polysialic acid product having alternating α2,9 - and α2,9 linkages of sialic acid. Contrary to the Examiner's allegation, the cited prior art does not disclose Step A of claim 7.

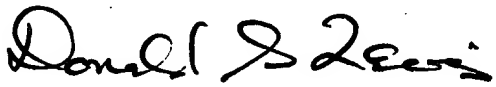
Within the field of enzymology, the catalytic formation by a single enzyme of two alternating glycosidic linkages to form a polymer is either unique or very unusual. In this connection, the precise interactions of an alkaline phosphatase with this mechanistically unusual enzyme, i.e., α2,9/α2,9 polysialyltransferase, and its impact on the synthetic process would have been entirely unpredictable. The present application teaches that the addition of alkaline phosphatase to the reaction mixture enhances the overall production of product by removing the CMP by-product so as to decrease inhibition of the forward reaction by CMP (Specification, page 16, first sentence and lines 15-20). It was patentably unobvious that the addition of alkaline phosphatase to a reaction mixture could enhance the yield of a catalytic synthesis that employed a mechanistically novel enzyme that operated to form alternating α2,9 - and α2,9 linkages of sialic acid.

Reversal of the final obvious rejection of claims 7-9 over Steenbergen or Vann in view of Van Dijk is respectfully requested.

Summary:

Applicant respectfully requests that the Board reverse Examiner's obviousness rejection of claims 7-9 and return the application to the Examiner for further processing.

Respectfully submitted,



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CLAIMS ON APPEAL

(Claims 7-9)

Listing of Claimson Appeal:

Claim 7. A method for synthesizing a polysialic acid product having alternating α2,9- and α2,8 linkages of sialic acid, said method comprising the following steps:

Step A: contacting a sialic acid acceptor and a CMP-sialic acid donor with α2,8/2,9 polysialyltransferase from *Escherichia coli* K92 under cell-free conditions for sequentially sialylating the sialic acid acceptor with the CMP-sialic acid donor for releasing CMP and forming the polysialic acid product; and

Step B: removing the released CMP with alkaline phosphatase.

Claim 8. A method according to claim 7 wherein the sialic acid acceptor is selected from the group consisting of sialic acid, an oligomer of sialic acid, and a ganglioside.

Claim 9. A method according to claim 9 wherein the sialic acid acceptor is selected from the group consisting of sialic acid, a dimer or trimer of sialic acid, *9-O*-acetyl sialic acid, GD_3, GM_3, GD_2, GT_{1a}, GT_{1b}, GQ_{1b}, and a ganglioside mixture.